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Prediction of rodent carcinogenic potential of naturally occurring chemicals in the human diet using high-throughput QSAR predictive modeling

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Abstract

Consistent with the U.S. Food and Drug Administration (FDA) Critical Path Initiative, predictive toxicology software programs employing quantitative structure–activity relationship (QSAR) models are currently under evaluation for regulatory risk assessment and scientific decision support for highly sensitive endpoints such as carcinogenicity, mutagenicity and reproductive toxicity. At the FDA's Center for Food Safety and Applied Nutrition's Office of Food Additive Safety and the Center for Drug Evaluation and Research's Informatics and Computational Safety Analysis Staff (ICSAS), the use of computational SAR tools for both qualitative and quantitative risk assessment applications are being developed and evaluated. One tool of current interest is MDL-QSAR predictive discriminant analysis modeling of rodent carcinogenicity, which has been previously evaluated for pharmaceutical applications by the FDA ICSAS. The study described in this paper aims to evaluate the utility of this software to estimate the carcinogenic potential of small, organic, naturally occurring chemicals found in the human diet. In addition, a group of 19 known synthetic dietary constituents that were positive in rodent carcinogenicity studies served as a control group. In the test group of naturally occurring chemicals, 101 were found to be suitable for predictive modeling using this software's discriminant analysis modeling approach. Predictions performed on these compounds were compared to published experimental evidence of each compound's carcinogenic potential. Experimental evidence included relevant toxicological studies such as rodent cancer bioassays, rodent anti-carcinogenicity studies, genotoxic studies, and the presence of chemical structural alerts. Statistical indices of predictive performance were calculated to assess the utility of the predictive modeling method. Results revealed good predictive performance using this software's rodent carcinogenicity module of over 1200 chemicals, comprised primarily of pharmaceutical, industrial and some natural products developed under an FDA-MDL cooperative research and development agreement (CRADA). The predictive performance for this group of dietary natural products and the control group was 97% sensitivity and 80% concordance. Specificity was marginal at 53%. This study finds that the *in silico* QSAR analysis employing this software's rodent carcinogenicity database is capable of identifying the rodent carcinogenic potential of naturally occurring organic molecules found in the human diet with a high degree of sensitivity. It is the first study to demonstrate successful QSAR predictive modeling of naturally occurring carcinogens found in the human diet using an external validation test. Further test validation of this software and expansion of the training data set for dietary chemicals will help to support the future use of such QSAR methods for screening and prioritizing the risk of dietary chemicals when actual animal data are inadequate, equivocal, or absent.
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Introduction

The use of computational-based quantitative structure–activity relationship (QSAR) modeling for predicting toxicity

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of organic molecular chemical entities from the pharmaceutical, food, and chemical industries has expanded in recent years with the evaluation of their use for regulatory purposes. Advanced predictive models are being designed and tested by regulatory agencies to assess physical, chemical, and biological properties of individual chemical entities using applications specific for decision-making frameworks in safety assessments. The use of QSAR modeling for toxicological predictions would help determine the potential adverse effects of chemical entities in risk assessment, chemical screening, and priority setting. The statistical-informatics QSAR approach to discerning the toxicological profile of a chemical would offer a powerful estimate of sensitive end points useful for safety evaluations of compounds under regulatory review. The technology is being developed with the goal of being able to quickly generate reliable, well validated, specific, and sensitive predictions that would meet today's challenges of estimating the carcinogenic potential of a vast number of compounds. With the effective use of archival test data obtained from numerous animal studies of similar chemicals, innovative software approaches for predicting chemical carcinogenicity and other toxicological endpoints could find applicability in addressing regulatory requirements or safety concerns during premarket development of natural and synthetic chemical entities. For example, computational methods can be useful when existing experimental data are insufficient, unreliable, unavailable, or inconsistent between studies. This approach reduces animal testing, facilitates the review process and also has applicability for the toxicological evaluation of chemically identified individual components of botanical mixtures, or chemicals of natural origin that have not been subjected to *in vivo* testing. This latter application is of particular relevance for materials from plant-derived, natural products that are difficult to synthesize by chemical means, or may be present in a complex mixture of such products (e.g., botanical extract), or are not the principal pharmacologically active constituent in a mixture and should still be considered for potential toxicological effects including carcinogenic risk.

Compounds discovered in nature show a much greater structural diversity and complexity than synthetic pharmaceuticals, and have utility as valuable single agents for therapeutic targets in clinical treatment of human disease conditions including cancer (Rowinsky and Donehower, 1995). Others have been found to modulate biological function through interaction with cellular macromolecules such as nuclear protein receptors, affecting expression of major enzymatic systems involved in xenobiotic metabolism (Choudhuri and Valerio, 2005). Many natural product chemicals are found as part of the human diet in conventional foods, spices, and flavoring agents at varying levels of concentration, resulting in a wide range of human dietary exposure. As a result of the variety of dietary sources and prevalence of natural products across different categories of food, there is a high human dietary exposure to small, organic chemicals of natural origin, and thus the concept of a diet free of potentially toxic naturally occurring chemicals is impossible. For example, it has been estimated that the human intake of natural toxicants may consist of 5000–10,000 different chemical species representing a daily average U.S. exposure of about 1.5–2.0 g from plants and burnt material from cooking (Gold et al., 2001). Increasingly, the vast background level of naturally occurring chemicals in the human diet is being recognized in cancer research, and an important element with respect to the influence of diet on cancer is the observation of the chronic exposure period inherent to the ingestion of food. The fact that natural products, especially those biosynthesized in plants, possess a broad spectrum of chemical functional groups, some of which are recognized as structural alerts for toxicologically-based chronic effects, such as the chemical initiation of carcinogenesis, portends to the importance of evaluating their potential toxicity and carcinogenicity. Such information may aid in addressing or mitigating concern for these widely prevalent chemicals. The challenge, however, emerges in that few natural products have been tested for systemic toxicological effects and even fewer have been tested in long-term rodent cancer bioassays. Given the potential for human exposure to the large number of synthetic and naturally occurring chemicals in discovery, development, or commercialization stages, there is a practical need for the development and validation of an efficient, reliable, and sensitive methodological approach for screening thousands of untested chemicals for toxicity. The necessity is especially apparent for highly sensitive endpoints such as carcinogenicity.

A key tool for screening such a diversity of compounds is the utilization of the "library" of pre-existing test data for use in a strategy of analyzing the characteristics of the chemical structural attributes of subject compounds, the results of previously conducted rodent carcinogenicity tests, and their molecular similarity to the compounds in the "library". Such a library has been recently developed and validated at the Food and Drug Administration, Center for Drug Evaluation and Research, Informatics and Computational Safety Analysis Staff (FDA/CDER ICSAS) for use in predicting the rodent carcinogenic potential of pharmaceuticals using *in silico* QSAR modeling (Contrera et al., 2003, 2005a; Matthews and Contrera, 1998).

Our interest was to evaluate the performance of the MDL-QSAR *in silico* modeling program for use in predicting the carcinogenic potential of naturally occurring chemicals found in the human diet. This category of compounds often lacks a complete battery of toxicological studies and it is possible that QSAR predictive modeling could be a useful tool for estimating the safety and potential carcinogenic risk of naturally occurring chemicals derived from plant-based food, botanical products, and medicinal plant-based remedies. Such an approach could have a wide range of applications in applied areas of toxicology such as regulatory and industrial toxicological safety assessment.

In the present study, the predictive performance of this software's discriminant analysis computational approach using its rodent carcinogenicity module (containing pharmaceutical, industrial and natural product chemicals) was evaluated using an external validation test set of naturally occurring and synthetic compounds. Results of the predictions were used to evaluate the performance of this software to accurately screen a sample set of 123 naturally occurring chemicals found in the human diet with known low and high risk potential as rodent

carcinogens, and a control group of 19 synthetic dietary chemicals with known high carcinogenic potential.

Materials and methods

Hardware and MDL-QSAR software program

The hardware used for the computational studies in this report was a PC with Microsoft Windows XP Professional version 2002. The QSAR modeling software program used in this investigation was MDL QSAR version 2.2 available from MDL Information Systems, San Ramon, CA (http://www.mdli.com) employing its rodent carcinogenicity module. The software program provides more than 240 physical–chemical, electrotopological E-state, connectivity and other descriptors, six algorithms to select from for conducting structure similarity searching and a variety of statistical methods for use in computational analysis involving both parametric and non-parametric methodologies. This combination of structural information on a diverse set of chemicals that have undergone long-term rodent *in vivo* testing for carcinogenic potential and the capability of structure similarity searching provide the basis for potentially excellent QSAR modeling of rodent carcinogenicity. A total of 52 descriptors were used in this software's rodent carcinogenicity module (Hall et al., 2006; Hall and Kier, 1999). The results were analyzed using a non-parametric discriminant analysis statistical method. Discriminant analysis classifies compounds into two categories as either "High" or "Low" risk for carcinogenicity. Carcinogenic potential is based on the molecular structure and the numeric classification for carcinogenic activity present in the learning data set. A description of the molecular and statistical features of the software are described in detail in previous reports (Kier and Hall, 1999; Contrera et al., 2005a).

MDL Rodent Carcinogenicity database

The MDL rodent carcinogenicity database employed in this study contained 1201 compounds with their names, identification codes, and molecular structures represented as 2D MDL.mole files. The chemicals represented in the carcinogenicity database are from FDA/CDER archives, the National Toxicology Program (NTP) study results, and the Lois Gold Carcinogenic Potency Database (CPDB). Approximately 25% of the compounds in the database are pharmaceuticals with the remaining data being industrial chemicals and a smaller number of dietary chemicals. A small number of natural product compounds are also present in the database. Most of the carcinogenicity study results for pharmaceuticals were derived from FDA/CDER pharmacology, toxicology, and biostatistics reviews. Results from FDA new drug application (NDA) studies for marketed pharmaceuticals are available under the Freedom of Information Act and are not considered proprietary. The identity and chemical structure of a small percentage of the pharmaceuticals contained in the database are not disclosed because they are currently under FDA regulatory review as investigational new drugs (IND), as new drug applications (NDA), or may represent drugs never approved for marketing, and therefore, are proprietary. The atom type E-state descriptors of coded proprietary pharmaceuticals were used to model carcinogenicity in this study and do not contain sufficient information to unambiguously reconstruct a proprietary molecular structure.

Data from FDA, NTP, and CPDB databases were collected and transformed into numeric representations of carcinogenic activity as previously described (Contrera et al., 2005a, 2003). Compounds that produce statistically significant tumors by pair-wise comparison at multiple organ/tissue sites in a single study cell (e.g., male or female group, rats or mice) were assigned a carcinogenic activity value of 50, the highest level. Compounds that produce statistically significant single site tumors were assigned a carcinogenic activity value of 40, and weaker or equivocal single site responses were assigned a value of 30 for carcinogenic activity. When studies found no statistically significant treatment-related tumor findings an activity value of 10 was assigned to the compound. Compounds with 30 or more activity units in 2 or more study cells were classified as high-risk carcinogens. Compounds below 30 activity units in 3 or more study cells were classified as low risk for carcinogenicity. When only one species was tested, compounds were considered positive if there were significant tumor findings in both sexes. Of the 1201 compounds in the database, more than 50% were classified as possessing high carcinogenic risk and the remaining compounds are classified as low carcinogenic risk. A similar multi-cell scoring method has been previously employed to predict rodent carcinogenicity of compounds for the MCASE QSAR computational platform (Matthews and Contrera, 1998). This transformed database is used as a training data set for MCASE and MDL-QSAR predictive software platforms that have been validated for screening pharmaceutical compounds (Contrera et al., 2003, 2005a).

Criteria for selection of dietary chemicals

General considerations. Natural and synthetic (control group) dietary chemicals were selected from, either the published literature or FDA files based on a weight of evidence (low and high) for their carcinogenic potential in rodents. The dietary chemicals were selected irrespective of the level of human exposure, any known mechanism of toxic action or target tissue for carcinogenicity. There are exclusion criteria for the molecular structures of chemicals that can be screened by this software. Generally, high molecular weight entities (>1000 MW), polymers, non-organic chemicals such as salts and fibers, organometallics, mixtures of organics, and molecules of very low molecular weight (<100 MW) are excluded from QSAR testing because they are outside the domain of applicability of this software's algorithm. All chemicals had to pass the computational inclusion criteria for testing by the QSAR software and criteria for sufficient molecular coverage with the MDL rodent carcinogenicity module as described below.

Molecular coverage. An important criterion in test screening a dietary chemical is to determine the coverage or representation of its molecular structure in the database. The membership-in-class statistic was used in the present study as criteria for molecular coverage because it can estimate the domain of applicability of a QSAR model. Previous studies employing this software to predict the carcinogenic and mutagenic potential of pharmaceuticals using discriminant analysis have employed the membership-in-class statistic (Contrera et al., 2005a, 2005b). Membership-in-class is defined as the probability that a chemical is associated with training set compounds that are either in the high or low risk categories (Contrera et al., 2005a). A more detailed description of membership-in-class and the selection of minimal membership values is presented in Contrera et al. (2005a). A minimum of 60% probability for membership-in-class was used in the classification of all compounds, and those that did not meet this probability limit were excluded. The basis for use of 60% as a threshold level for membership-in-class is from validation experiments with pharmaceuticals that found validation test compounds with membership-in-class statistics in the 60% range may be considered adequately covered by the software's database (Contrera et al., 2005a). Whereas values below 60% were considered to be an inadequate molecular coverage (Contrera et al., 2005a). In external validation, the predictive performance of a QSAR model is evaluated using validation test compounds that were never part of the training data set and ideally should be a completely independent data set. In an internal validation study, test compounds are randomly removed from the training data set before a QSAR model is generated. The predictive performance of the model is then evaluated using the randomly removed test set.

In addition, in assessing the suitability of external-validation test compounds, it was confirmed that the compounds were not present in the rodent carcinogenicity-training database.

Selection of naturally occurring dietary chemicals. Three general criteria were set for entrance of a naturally occurring dietary chemical into the study: (1) the chemical had to have a known natural occurrence, (2) human oral exposure from conventional food or from a herbal, botanical extract, or medicinal plant remedy, and (3) a preferential background of long-term testing of rodent carcinogenic potential which could be available from classifiable standardized studies at the National Toxicology Program (NTP), the International Agency for Research on Cancer (IARC), the Gold Carcinogenic Potency Database (CPDB), studies from the FDA/CFSAN Priority Assessment of Food Additives Database (PAFA), or from non-standardized rodent cancer bioassays published in the open scientific literature.

The naturally occurring chemicals meeting the above criteria were found at random from the open literature and aforementioned databases. Particular

attention was given to identifying chemicals with associated experimental toxicological studies useful for verifying carcinogenic potential.

The majority of chemicals in this group were derived from plants, and are known constituents in the human diet. These chemicals are found in a wide range of human dietary sources including, conventional food (whole food stuffs, condiments, and teas), flavors, spices, herbal remedies, and by products produced during cooking. The chemicals for the QSAR screening were selected from each of these dietary sources in order to achieve a diversity of natural compounds. However, a quantitative approach in the selection of chemicals from each dietary source was not employed in this study. The identification of the natural occurrence of chemicals by plant source (*Genus, species*) was confirmed using the published literature and phytochemical databases (Duke, 2006).

Several other naturally occurring carcinogens that have been well recognized as human health hazards, but were not included in our predictive analysis, include fungal toxins and their metabolites such as the aflatoxin family of compounds. The reason for exclusion of these types of natural compounds in this study is that these potent carcinogens are unintentional, opportunistic contaminant species of food. Whereas, the focus of this study was primarily naturally occurring chemicals that are biosynthesized by whole plants and have a constitutive presence in the human diet. Nevertheless, some compounds such as the heterocyclic amines which are produced during over cooking of meat were included in order to test a widely recognized class of rodent carcinogens that are found at comparatively high concentrations in the human diet.

If a given naturally occurring dietary chemical had not been tested in any available long-term animal cancer bioassay, then the peer-reviewed scientific literature regarding mechanism of toxicity and other published toxicological profiling served as a means to assess carcinogenic risk based on the totality of the experimental evidence as detailed in the following section Assessment of experimental evidence of carcinogenic potential.

Selection of a control group of dietary chemicals. Nineteen known carcinogenic dietary chemicals of synthetic origin served as a control group (Table 2). Selection of this group of chemicals was made from 75 known carcinogenic constituents obtained from CFSAN files. Fifty-six of the 75 carcinogens were found to be present in the MDL training set leaving 19 suitable for testing as a control group. The testing of these chemicals as controls enables an assessment of the predictive performance of the rodent carcinogenicity software module for synthetic dietary compounds because of the availability of robust experimental data on their carcinogenic potential, and the fact they are synthetic in origin as are the majority of chemicals in the database of the software module. These synthetic compounds possess well characterized rodent carcinogenic potency and had acceptable molecular coverage within the database of the software. The selection of the 19 controls for QSAR testing was irrespective of target organs for tumor formation. The majority of these chemicals are found in the human diet as contaminants, and therefore, their concentrations in food would be expected to be similar to many of the natural dietary chemicals included in the study.

Assessment of experimental evidence for carcinogenic potential

The criteria used in the assessment of the experimental evidence for carcinogenic potential of a naturally occurring chemical was based upon a "weight of evidence" evaluation of experimental findings published in the literature, rodent 2-year bioassay studies, or data and conclusions published in the NTP, IARC, PAFA and Gold CPDB databases. If the evidence for or against carcinogenic activity for a chemical was derived from a chronic rodent cancer bioassay published in the open scientific literature, then the study was used and could supersede other experimental evidence (e.g., negative or positive *in vitro* genetic toxicology studies). Any positive or negative finding from a published chronic rodent cancer bioassay was based on the author's opinion in the published paper. In addition, the carcinogenic potential of naturally occurring chemicals was evaluated using Ashby–Tennant structural alerts (Ashby and Tennant, 1991). For naturally occurring chemicals lacking rodent cancer bioassay results, Ashby–Tennant structural alerts for mutagenicity were used to classify these chemicals as high carcinogenic risk. The presence of Ashby–Tennant alerts in a compound is considered an indicator of a potential carcinogen although the absence of such alerts cannot be used to classify a chemical as a non-carcinogen (Ashby and Tennant, 1991). Regulatory agencies recommend that chemicals be evaluated for genotoxicity, which is based upon exposure levels and the potential for positive mutagens to be also carcinogens (McCann et al., 1975; Tennant et al., 1987; Zeiger et al., 1990). Compounds lacking sufficient experimental evidence and structural alerts were excluded from this study.

External validation experiment

In conducting an external validation study, the predictive performance of a QSAR model is evaluated using test compounds that were not part of the database learning set used to generate the QSAR model. External validation is the best way to determine the predictivity and reliability of a QSAR model (Perkins et al., 2003; Golbraikh and Tropsha, 2002). In this study, a total of 142 dietary chemicals (123 natural, 19 synthetic) that were completely independent and never part of the MDL rodent carcinogenicity database of 1201 compounds were used as the external validation test set. This is considered to be the most rigorous type of validation (Eriksson et al., 1997, 2003).

Statistical analysis of predictive performance

Predictive performance indices for the QSAR model were calculated as previously described according to the method of Cooper et al. (Cooper et al., 1979). This method of measuring performance is designed for evaluating data obtained from chemical carcinogen screening. The statistical parameters of interest are sensitivity, specificity, positive predictivity, negative predictivity, false positive rate, false negative rate, and concordance. Sensitivity is defined as the percentage of correctly classified carcinogens among the total number of carcinogens. Specificity is the percentage of correctly classified non-carcinogens among the total number of non-carcinogens. Positive predictivity is the percentage of correctly classified carcinogens among the total number of positive predictions from the test. Negative predictivity is the percentage of correctly classified non-carcinogens among the total number of negative predictions from the test. False positive prediction rate is the percentage of incorrectly classified carcinogens among the total number non-carcinogens. False negative prediction rate is the percentage of incorrectly classified non-carcinogens among the total number of carcinogens. Concordance is defined as the total number of non-carcinogens and carcinogens correctly predicted among the total number of compounds tested.

Results

Table 1 lists the chemical names and natural occurrence for the complete set of 123 naturally occurring chemicals tested using the software. The natural occurrence listed for each compound is not, in many cases, exclusive to that source. For example, anethole is listed as occurring in fennel, but it is also known to occur in germander, licorice, and peppermint (Duke, 2006). Other compounds listed are novel (*N*-benzylhexadecanamide) and are only known to occur in the plant indicated (Valerio and Gonzales, 2005), or are widely prevalent within a genus (*Aristolochia*; aristolochic acid I) (De Smet, 1992).

A control set of synthetic compounds with rodent carcinogenicity results is listed in Table 2. A minimum of 60% probability of membership-in-class was used in the classification of all compounds. This threshold of probability is considered to be the lowest acceptable value for adequate representation and coverage of a molecule with the compounds in the database of the software. Those that did not meet this probability limit are listed as NC (not covered) for their prediction. Based on these modeling data, 6 out of the 19 synthetic rodent carcinogens did not meet the minimum 60% probability-in-class ("high" or "low"), and therefore, these compounds were

Table 1
123 Naturally occurring dietary chemicals screened for rodent carcinogenic potential using MDL-QSAR discriminant analysis modeling

Chemical	Natural occurrence
1-Hydroxyanthraquinone	Medicinal herb, *Morinda officinalis*
1′-Hydroxyestragole	Basil, *Ocimum basilicum;* Metabolite of estragole
1-Octacosanol	Perilla seed, *Perilla frutescens*
2-Amino-1-methyl-6-phenylimidazo [4,5-b]pyridine	Heterocyclic amine from overcooked meat, PhIP
2-Amino-6-methyldipyrido [1,2-*a*:3′,2′-d]imidazole	Heterocyclic amine from overcooked meat, Glu-P-1
2-Ethyl-1-hexanol	Sassafras, *Sassafras albidum;* Metabolite of safrole
2-Hexenal	Banana, *Musa acuminate*
2-Pentanone	Banana, *Musa acuminate*
3,7-dimethyl-2,6-octadienol (Geraniol)	Orange, *Citrus sinsensis*
4-Anisaldehyde	Fennel, *Foeniculum vulgare*
4-methylphenylhydrazine	Edible mushroom, *Agaricus bisporus*; metabolite
5-Methoxypsoralen	Parsley, *Petroselinum sativum*
6,7-Dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine	Riddell's groundsel *Senecio riddellii*
6-Methylcoumarin	Oregano, *Origanum vulgare*
7-Acetylintermedine	Honey and Ragwort herb, *Senecio jacobaea*
7-Acetyllycopsamine	Common Comfrey, *Symphytum Officinale*
Acetylechimidine	Honey and Ragwort herb, *Senecio jacobaea*
Allyl 3-cyclohexylpropionate	Pineapple, *Ananas comosus*
Allyl hexanoate	Tea Tree oil, *Melaleuca alternifolia*; flavor
Anethole	Fennel, *Foeniculum vulgare*
Anise alcohol	Anise seed, *Pimpinella anisum*
Aristolochic acid I	Virginia Snake root, *Aristolochia sp.*
Aristolochic acid II	Virginia Snake root, *Aristolochia sp.*
Auraptene (7-geranyloxycoumarin)	Lemon, *Citrus limon*
N-Benzylhexadecanamide	Maca, *Lepidium meyenii*
Berberine	Goldenseal, *Hydrastis Canadensis*
Beta-apo-8′-carotenal	Carrot, *Paucus carota*
Capsaicin	Hot pepper, *Capsicum annum*
Carnosic acid	Rosemary, *Rosmarinus officinalis*
Carnosol	Rosemary, *Rosmarinus officinalis*
Catechol	Roasted coffee, *Coffea arabica*
α-Chaconine	Potato, *Solanum tuberosum*
Chlorogenic acid	Roasted coffee, *Coffea Arabica*
Cichoric acid	Echinacea, *Echinacea purpurea*
Cimicifugic acid A	Black cohosh, *Actae racemosa*
Citrate	Lemon, *Citrus limon*
Citronellol	Ginger, *Zingiber officinale*
Crotonaldehyde	Potato, *Solanum tuberosum*
Curcumin	Turmeric, *Curcuma longa*
Cycasin	Cycad seed, *Cyas circinalis*
Decursin	Nakai (Korean) root/medicinal tonic, *Angelica gigas*
Decursinol	Nakai (Korean) root/medicinal tonic, *Angelica gigas*
Dehydromonocrotaline	Russian comfrey, *Symphytum uplandicum*
Dihydromethysticin	Kava Kava, *Piper methysticum*
Echimidine	Russian comfrey, *Symphytum uplandicum*
Elemicin	Nutmeg, *Myristica fragrans*
Ellagic acid	Strawberry fruit, *Fragaria* spp.
Epicatechin	Green Tea, *Camellia sinensis*
Estragole	Basil, *Ocimum basilicum*
Ethyl vanillin	Vanilla, *Vanilla planifolia*
Falcarinol	Carrot, *Paucus carota*
Formic acid	Carrot, *Paucus carota*
Formononetin	Black cohosh, *Actae racemosa*
Gallic acid	Mango, *Mangifera indica*
Glabridin	Licorice, *Glycyrrhiza glabra*
Heliotrine	Russian comfrey, *Symphytum uplandicum*
Hexanal	Orange, *Citrus sinensis*
Hydroxychavicol	Sassafras, *Sassafras albidum*
Hydroxymethylphenylhydrazine	Edible mushroom, *Agaricus bisporus*; metabolite
Hydroxysenkirkine	Medicinal herb, *Crotalaria laburnifolia*
Imperatorin	Oregano, *Origanum vulgare*
Indole	Corn, *Zea mays*
Indole-3-acetic acid	Strawberry fruit, *Fragaria vesca*
Indole-3-carbinol	Broccoli, *Brassica oleracea*
Intermedine	Common Comfrey, *Symphytum officinale*
Ipomeamarone	Sweet potato, *Ipomoea batatas*
Isobutylamide	Echinacea, *Echinacea purpurea*
Isopimpinellin	Oregano, *Origanum vulgare*
Isopteropodine	Cat's claw, *Uncaria tomentosa*
Isosafrole	Oil of Sassafras, *Sassafras albidum*
Jacobine	Ragwort herb, *Senecio jacobaea*
Jacoline	Honey and Ragwort herb, *Senecio jacobaea*
Jaconine	Honey and Ragwort herb, *Senecio jacobaea*
Jacozine	Honey and Ragwort herb, *Senecio jacobaea*
Juglone	Walnuts, *Juglans mandshurica*
Kawain	Kava Kava, *Piper methysticum*
Limonin	Grapefruit, *Citrus paradis*
Linalool	Apricots, *Prunus armeniaca*
Lipoic acid	Spinach, *Spinacia oleracea*
L-Theanine	Green Tea, *Camellia sinensis*
Lycopsamine	Common comfrey, *symphytum officinale*
Maltol	Roasted coffee, *Coffea Arabica*
Methyl salicylate	Wintergreen leaf, *Gaultheria procumbens*
Methylglyoxal	Roasted coffee, *Coffea Arabica*
Methysticin	Kava Kava, *Piper methysticum*
Myristicin	Parsley, *Petroselinum sativum*
Naringin	Grapefruit, *Citrus paradis*
Neochlorogenic acid	Roasted coffee, *Coffea Arabica*
Oleuropein	Olive oil, *Olea europaea*
Oxalic acid	Spinach, *Spinacia oleracea*
Paeonol	Peony root bark, *Paeonia suffruticosa*
Parasorbic acid	Rowan berry, *Sorbus aucubaria*
Piperine	Black pepper, *Piper nigrum*
Pipermethystine	Kava Kava, *Piper methysticum*
Piperonal	Vanilla, *Vanilla planifolia*
Propionic acid	Tomato, *Lycopersicon esculentum*
Protocatechuic acid	Shallot onions, *Allium cepa*
Ptaquilosin	Bracken fern, *Pteridum aquilinum*
Ptaquilosin (APT) dienone	Bracken fern, *Pteridum aquilinum*
Ptaquilosin B	Bracken fern, *Pteridum aquilinum*
Pulegone	Pennyroyal Mint, *Mentha Pulegium*

(continued on next page)

Table 1 (*continued*)

Chemical	Natural occurrence
Resveratrol	European Grapes, *Vitis vinifera*
Retronecine	Medicinal herb, *Crotalaria laburnifolia*
Riddelliine *N*-oxide	Riddell's groundsel *Senecio riddellii*
Rosmarinic acid	Rosemary, *Rosmarinus officinalis*
Rutin trihydrate	Tea, *Camellia sinensis*
Senecionine	Honey and Ragwort herb, *Senecio jacobaea*
Seneciphyllinine	Honey and Ragwort herb, *Senecio jacobaea*
Sesamol	Sesame oil, *Sesamum orientale*
Silibinin	Milk Thistle, *Silybum marianun*
β-Sitosterol	Roman coriander, *Nigella sativa*; ubiquitous
Sulforafan	Cabbage, *Brassica oleracea*
Symphytine	Russian comfrey, *Symphytum uplandicum*
Synephrine-*para*	Bitter orange, *Citrus aurantium*
Tannic acid	Tea, *Camellia sinensis*
Taspine	Dragon's blood, *Croton lecheri*
Teuchamaedryn A	Germander, *Teuchrium chamaedrys*
Teucrin A	Germander, *Teuchrium chamaedrys*
α-Thujone	Wormwood, *Artemisia absinthium*
Uplandicine	Honey and Ragwort herb, *Senecio jacobaea*
Ursolic acid	Apple, *Malus domestica*; ubiquitous
Valerenic acid	Valerian, *Valeriana officinalis*
Vanillin	Vanilla, *Vanilla planifolia*

considered poorly represented in the learning data set and not included in performance statistics.

Table 3 summarizes the predictions for the classification of the carcinogenic risk of the naturally occurring dietary chemicals. As with the control group compounds, a minimum of 60% probability of membership-in-class was used in the classification. Those which did not meet this probability limit were not included in the table and were also not included in the statistical performance results in Table 4. For ease of comparison to the software's predictions, we have also provided the experimental evidence-based risk derived from animal studies and/or Ashby–Tennant structural alert classification for each chemical. Citations supporting the classification of each chemical based on experimental evidence are also listed in Table 3. The naturally occurring chemicals listed in Table 3 are divided into two groups; those with and those without a chronic rodent carcinogenicity study. Chemicals which were tested in an anti-carcinogenicity study such as dietary feeding prior to administration of a known synthetic carcinogen to test for inhibition of tumors were listed with the group of compounds as not having a chronic rodent carcinogenicity study (e.g., resveratrol).

It was determined that of the 142 natural and control group dietary chemicals screened, 114 had met the minimum criteria of 60% probability of membership-in-class. Therefore, their molecular structures were adequately covered by the rodent carcinogenicity training database. The predictive performance statistics listed in Table 4, included the 114 qualifying natural and control group dietary chemicals. Approximately 55% of the naturally occurring chemicals with adequate molecular coverage were known to be rodent carcinogens based on results of long-term rodent bioassay testing, or there was experimental evidence published in the scientific literature highly suggestive of potential carcinogenic activity or they contained structural alerts for genotoxicity and mutagenicity.

MDL-QSAR made correct predictions for 97% of the naturally occurring chemicals with experimental high risk rodent carcinogenic potential. The modeling data for the remaining naturally occurring chemicals with experimentally-based low risk rodent carcinogenic potential demonstrated that MDL-QSAR made correct predictions for 53% of the qualified compounds. When considering only naturally occurring chemicals with chronic rodent carcinogenicity data (Table 3 first group), approximately 93% were correctly predicted to be of "high" carcinogenic risk, and 55% were correctly predicted to be of "low" carcinogenic risk.

Discussion

The results of this study suggest that MDL discriminant analysis may be useful for predicting the carcinogenic potential of natural and synthetic dietary chemicals. In addition, it may be useful as a decision support tool in a regulatory environment and a research prioritization tool for industry. It may be especially useful in situations where there is a paucity of experimental evidence, and a lack of extensive toxicity testing, as is often the case with natural products, especially those originating from plants. The absence of experimental evidence is more apparent for long-term studies such as rodent carcinogenicity studies, than shorter-term tests such as genotoxicity. Genotoxicity can, in some cases, afford reasonable predictivity of carcinogenicity in rodents if the chemical

Table 2
External validation MDL-QSAR prediction results for rodent carcinogens of the control group

Synthetics group	Low risk probability	High risk probability	MDL-QSAR predicted risk
1-Methyl-2-pyrrolidinone	0.2838	0.7162	High
2-Ethyl-1-hexanol	0.2802	0.7198	High
2-Nitropropane	0.2019	0.7982	High
4,4′-Diphenylmethanediamine	0.3796	0.6204	High
4-Aminoazobenzene	0.3955	0.6045	High
Acetaldehyde	0.1947	0.8053	High
Allyl glycidyl ether	0.1062	0.8938	High
Dibutyltin diacetate	0.0000	1.0000	High
N-vinyl-2-pyrrolidone	0.1592	0.8408	High
Permanent orange	0.1026	0.8975	High
Quinoline	0.3309	0.6691	High
Tri-n-butyl phosphate	0.0945	0.9055	High
Ammonium perfluorooctanoic acid	1.0000	0.0000	Low
1,3-Dichloropropanol	0.4864	0.5137	NC
3-Chloro-1,2-propanediol	0.5892	0.4108	NC
4-Aminobiphenyl	0.4668	0.5332	NC
4-Hydroxyphenylacetamide	0.5090	0.4910	NC
Diazoaminobenzene	0.4393	0.5607	NC
Sudan I	0.4186	0.5814	NC

NC = not covered.

Table 3
Concordance between experimental evidence-based risk assessment and external validation MDL-QSAR predictions for rodent carcinogenic potential of 101 naturally occurring chemicals found in the human diet

Naturally occurring chemical	Experimental evidence-based carcinogenic risk	Reference	MDL-QSAR prediction of carcinogenic risk	Concordance (√) or Non-concordance (−) of MDL-QSAR prediction with experimental evidence
Chemicals tested in a chronic rodent carcinogenicity study				
1′-Hydroxyestragole	High	(Wakazono et al., 1998; Gold et al., 2005; Drinkwater et al., 1976)	High	√
1-Octacosanol	Low	(Aleman et al., 2005)	Low	√
2-Amino-1-methyl-6-phenylimidazo[4,5-b] pyridine	High	(IARC, 1993; Sugimura, 1997; Turesky, 2002; National Toxicology Program, 2005)	High	√
2-Amino-6-methyldipyrido [1,2-*a*:3′,2′-d]imidazole	High	(Sugimura, 1997)	High	√
2-Ethyl-1-hexanol	High	(Gold et al., 2005)	High	√
4-Methylphenylhydrazine	High	(Toth et al., 1977; Levenberg, 1964)	High	√
5-Methoxypsoralen	High	(Zajdela and Bisagni, 1981; IARC, 1998a)	High	√
6-Methylcoumarin	High	(Ashby and Tennant, 1991; Cramer et al., 1978; Hagan et al., 1967; Ronchi and Arcara, 1967)	High	√
7-Acetyllycopsamine	High	(Fu et al., 2004; Mei et al., 2005; Hirono et al., 1978)	High	√
Allyl hexanoate	Low	(Hagan et al., 1967)	High	−
Anethole	Low	(Gold et al., 2005; Wiseman et al., 1987; Mortelmans and Haworth, 1986)	High	−
Aristolochic acid I	High	(Arlt et al., 2002; Nortier et al., 2000)	High	√
Aristolochic acid II	High	(Arlt et al., 2002; Nortier et al., 2000)	High	√
Beta-apo-8′′-carotenal	Low	(Rulis et al., 1984)	High	−
Capsaicin	High	(Gold et al., 2005; Surh and Lee, 1996)	Low	−
Citrate	Low	(Gold et al., 2005)	High	−
Crotonaldehyde	High	(Gold et al., 2005; Chung et al., 1986)	High	√
Curcumin	Low	(Gold et al., 2005; Rulis et al., 1984)	Low	√
Dehydromonocrotaline	High	(Mattocks and Cabral, 1982)	High	√
Epicatechin	Low	(Gold et al., 2005)	Low	√
Estragole	High	(Drinkwater et al., 1976; Gold et al., 2005; Anthony et al., 1987; Wakazono et al., 1998)	High	√
Formic acid	Low	(Rulis et al., 1984)	Low	√
Gallic acid	Low	(Gold et al., 2005; Shibata et al., 1993)	Low	√
Heliotrine	High	(Schoental, 1975)	High	√
Hydroxymethylphenylhydrazine	High	(Levenberg, 1964; Toth and Nagel, 1978)	High	√
Hydroxysenkirkine	High	(Schoental and Cavanagh, 1972)	High	√
Indole	Low	(Gold et al., 2005)	Low	√
Indole-3-acetic acid	Low	(Gold et al., 2005; Chemical Carcinogenesis Research Information System, 2006a)	Low	√
Intermedine	High	(Prakash et al., 1999; Schoental et al., 1970, 1971)	High	√
Isosafrole	High	(IARC, 1976c; Hagan et al., 1967, 1965)	High	√
Jacobine	High	(Petry et al., 1986; Mori et al., 1985; IARC, 1976a; Cook et al., 1950; Schoental et al., 1954)	High	√
Linalool	Low	(Stoner et al., 1973; Heck et al., 1989)	High	−
Lipoic acid	Low	(Ames, 1998; Cremer et al., 2006)	Low	√
Lycopsamine	High	(Fu et al., 2004; Hirono et al., 1978; Schoental et al., 1970, 1971)	High	√
Maltol	Low	(Rulis et al., 1984)	High	−
Methylglyoxal	High	(Fujita et al., 1984)	High	√
Parasorbic acid	High	(IARC, 1976b)	High	√
Piperine	Low	(Wiseman et al., 1987; Pradeep and Kuttan, 2002; Selvendiran et al., 2004)	High	−
Piperonal	Low	(Hagan et al., 1967)	High	−
Propionic acid	High	(Rulis et al., 1984)	High	√
Protocatechuic acid	Low	(Hirose et al., 1995; Nakamura et al., 2000; Hirose et al., 1992; Chung et al., 1986; Tanaka et al., 1993b; Tanaka et al., 1993a, 1994)	Low	√
Ptaquilosin	High	(IARC, 1998b; Hirono et al., 1970; Potter and Baird, 2000; Shahin et al., 1999)	High	√
Ptaquilosin (APT) dienone	High	(Shahin et al., 1998a, 1998b)	High	√
Retronecine	High	(Schoental and Cavanagh, 1972)	Low	−

(continued on next page)

Table 3 (*continued*)

Naturally occurring chemical	Experimental evidence-based carcinogenic risk	Reference	MDL-QSAR prediction of carcinogenic risk	Concordance (√) or Non-concordance (−) of MDL-QSAR prediction with experimental evidence
Chemicals tested in a chronic rodent carcinogenicity study				
Rutin trihydrate	Low	(Yu et al., 2005; Romert et al., 1994; Chemical Carcinogenesis Research Information System, 2006b; Habs et al., 1984)	Low	√
Senecionine	High	(Prakash et al., 1999; Schoental and Head, 1957; Schoental et al., 1954; Hirono et al., 1976)	High	√
Seneciphyllinine	High	(Schoental et al., 1954; Schoental and Head, 1957)	High	√
Symphytine	High	(Hirono et al., 1978, 1979)	High	√
Tannic acid	Low	(Nagabhushan et al., 1991; Rulis et al., 1984; Onodera et al., 1994)	Low	√
Vanillin	Low	(Hagan et al., 1967)	High	−
Chemicals not tested in a chronic rodent carcinogenicity study				
2-Hexenal	High	(Nadasi et al., 2005; Golzer et al., 1996; Eder and Schuler, 2000; Eder et al., 1999)	High	√
2-Pentanone	High	(Hewitt et al., 1983; Brown and Hewitt, 1984; Pitarque et al., 1999)	High	√
4-Anisaldehyde	High	(Feron et al., 1991; Becker et al., 1996)	High	√
7-Acetylintermedine	High	(Prakash et al., 1999)	High	√
7-Acetyllycopsamine	High	(Prakash et al., 1999)	High	√
Acetylechimidine	High	(Prakash et al., 1999)	High	√
Auraptene (7-geranyloxycoumarin)	High	(Sakata et al., 2004; Kohno et al., 2006; Ashby and Tennant, 1991)	High	√
N-Benzylhexadecanamide	Low	(Gonzales and Valerio, 2006; Valerio and Gonzales, 2005)	Low	√
Berberine	Low	(Anis et al., 2001)	High	−
Carnosic acid	Low	(Sharabani et al., 2006; Offord et al., 2006; DelBano et al., 2006)	Low	√
Carnosol	Low	(Moran et al., 2005)	Low	√
α-Chaconine	Low	(Sharma et al., 1983; Friedman et al., 1996; Friedman and Henika, 1992)	High	−
Chlorogenic acid	High	(Hagiwara et al., 1991; Stich et al., 1981b; Stich et al., 1981a; Ashby and Tennant, 1991)	High	√
Cichoric acid	Low	(Birosova et al., 2005; Pellati et al., 2006; Matthias et al., 2005)	Low	√
Cimicifugic acid A	Low	(Burdette et al., 2002)	High	−
Decursin	High	(Ashby and Tennant, 1991)	High	√
Decursinol	High	(Ashby and Tennant, 1991)	High	√
Echimidine	High	(Eroksuz et al., 2001; Prakash et al., 1999)	High	√
Elemicin	High	(Ashby and Tennant, 1991; Phillips et al., 1984)	High	√
Falcarinol	Low	(Kobak-Larsen et al., 2005)	Low	√
Hexanal	High	(Brambilla et al., 1989; Martelli et al., 1994)	High	√
Imperatorin	Low	(Prince et al., 2006)	High	−
Indole-3-carbinol	Low	(Yu et al., 2006; Shertzer and Senft, 2000)	Low	√
Intermedine	High	(Hirono et al., 1978)	High	√
Isopimpinellin	Low	(Prince et al., 2006)	High	−
Isopteropodine	Low	(Valerio and Gonzales, 2005)	High	−
Jacoline	High	(Prakash et al., 1999)	High	√
Jaconine	High	(Prakash et al., 1999)	High	√
Jacozine	High	(Prakash et al., 1999)	High	√
Juglone	High	(National Library of Medicine, 1999; Monks et al., 1990; Tikkanen et al., 1983)	High	√
Kawain	High	(Ashby and Tennant, 1991)	High	√
Limonin	Low	(Vanamala et al., 2006; Tanaka et al., 2000)	High	−
Methysticin	High	(Ashby and Tennant, 1991)	High	√
Myristicin	Low	(Hasheminejad and Caldwell, 1994)	High	−
Naringin	Low	(Vanamala et al., 2006; So et al., 2006)	High	−
Neochlorogenic acid	High	(Ashby and Tennant, 1991; Gold et al., 2001)	High	√
Oleuropein	Low	(Hamdi and Castellon, 2005)	Low	√
Oxalic acid	Low	(McMartin and Wallace, 2005; Miller et al., 2000; DePass et al., 1986; National Toxicology Program, 1993)	Low	√
Ptaquilosin B	High	(Shahin et al., 1999; Potter and Baird, 2000; IARC, 1998b)	High	√

Table 3 (*continued*)

Naturally occurring chemical	Experimental evidence-based carcinogenic risk	Reference	MDL-QSAR prediction of carcinogenic risk	Concordance (√) or Non-concordance (−) of MDL-QSAR prediction with experimental evidence
Chemicals not tested in a chronic rodent carcinogenicity study				
Resveratrol	Low	(Li et al., 2002; Whitsett et al., 2006; National Toxicology Program, 2002; Horn et al., 2007)	Low	√
Riddelliine *N*-oxide	High	(Wang et al., 2004; Fu et al., 2004; Chou et al., 2003)	High	√
Rosmarinic acid	Low	(Renzulli et al., 2004)	High	−
Seneciphyline	High	(Prakash et al., 1999; Schoental and Cavanagh, 1972)	High	√
Silibinin	Low	(Singh et al., 2006; Mallikarjuna et al., 2004)	Low	√
β-Sitosterol	Low	(Beier, 1990)	High	−
Sulforafan	Low	(Thejass and Kuttan, 2006; Zhang et al., 1994)	High	−
para-Synephrine	Low	(National Toxicology Program, 1987)	Low	√
Taspine	High	(Gonzales and Valerio, 2006)	High	√
Uplandicine	High	(Prakash et al., 1999)	High	√
Ursolic acid	Low	(Beier, 1990)	Low	√
Valerenic acid	Low	(Block et al., 2004)	Low	√

mechanism of action involves DNA modification (Tennant et al., 1990). Validated predictive QSAR software for carcinogenicity can offset the need for costly and resource intensive 2-year rat or mouse cancer bioassays for some applications. The financial loss in pharmaceutical drug development for a failed drug due to carcinogenicity findings can be even greater due to the many years lost in the development effort (Grabowski et al., 2000). In the case of industrial chemicals and natural products, few are tested in a full 2-year bioassay in both sexes and 2-species. Additionally, due to the high cost and labor intensiveness, a limited number of compounds are nominated by regulatory agencies for further animal testing by the National Toxicology Program and fewer of these are accepted for testing each year. In general, results of such studies are not available for at least 5 years. These considerations and improvements in informatics and computer technology are attracting greater attention to the development of valid alternative methodologies worldwide (OECD, 2006a, 2006b; Van Der JAGT et al., 2003; REACH, http://ecb.jrc.it/REACH/).

At the US Food and Drug Administration (FDA), Office of Food Additive Safety, a "weight of evidence" approach using structure–activity relationship analysis of food contact substances (FCS) is already being used during the safety evaluation of FCSs (Bailey et al., 2005). The FDA, Center for Drug Evaluation and Research (CDER), Office of Pharmaceutical Science (OPS), Informatics and Computational Safety Analysis Staff (ICSAS) is an applied regulatory research unit that compiles toxicology and safety related databases as a toxicological information resource for the Agency (http://www.fda.gov/cder/Offices/OPS_IO/default.htm). ICSAS also produces databases suitable for quantitative structure-activity (QSAR) modeling and uses these transformed databases to develop toxicology prediction software and to evaluate commercial QSAR, SAR, and data mining software to meet the needs of the FDA, other regulatory agencies, and the scientific community. These efforts are accomplished through research collaborations with software developers using leveraging arrangements such as Material Transfer Agreements (MTAs) and Cooperative Research and Development Agreements (CRADAs). ICSAS' mission is to develop a complete battery of predictive software for all of the major toxicology studies recommended by the FDA's Centers. The software can be used to: (1) improve lead compound selection by identifying and eliminating compounds with potentially significant adverse properties early in the drug discovery and development process; (2) reduce the use of animals in testing by eliminating non-critical laboratory studies; (3) facilitate and accelerate the review process by making better use of accumulated scientific knowledge (regulatory decision support); and (4) expand the role of predictive toxicology by encouraging the development of complementary predictive software systems through collaboration with software developers and the scientific community.

Table 4
Predictive performance of MDL-QSAR for rodent carcinogenic potential of naturally occurring and control group dietary chemicals

Performance parameters	Percent (%)
Sensitivity	97
Specificity	53
Positive predictivity	77
Negative predictivity	89
False positive rate	47
False negative rate	4
Concordance	80

Given this keen interest in finding a validated and reliable technique for predicting the carcinogenic potential of a chemical, and because of the aforementioned gaps in long-term toxicity testing that exists for many naturally occurring chemicals, there is impetus for screening this class of chemicals using QSAR methodology such as the MDL rodent carcinogenicity module. This software was of interest to us for testing and externally validating the utility of this approach with respect

to natural and synthetic dietary chemicals. The modeling results for this experiment demonstrated exceptional performance of the program in predicting rodent carcinogenic potential for both naturally occurring (Table 3) and the control group of dietary chemicals (Table 2) with 97% sensitivity, concordance of 80% and a low percentage (4%) of false negative predictions (Table 4). The modeling, however, did not perform as well in predicting non-carcinogens with only 53% specificity (Table 4). From a regulatory perspective, the higher sensitivity in predicting carcinogens is more desirable than high specificity due to regulatory conservatism and statutory requirements under provisions of the Federal Food, Drug, and Cosmetic Act.

Evaluation of the predictive performance for the group of naturally occurring chemicals that have chronic rodent carcinogenicity studies found that the MDL-QSAR predictive modeling approach performed exceptionally well. For this group, 93% were correctly predicted to be of "high" carcinogenic risk, and 55% were correctly predicted to be of "low" carcinogenic risk.

Likewise, modeling of the control group resulted in the correct prediction of "high" carcinogenic risk for 12 out of the 13 chemicals found to have adequate coverage with the carcinogenicity training database (Table 2). The only anomaly in this group was the prediction of 100% probability that ammonium perfluorooctanoic acid would be of "low" carcinogenic risk, when in fact it is a rodent carcinogen. The reason for the erroneous prediction could be due to the low representation of highly halogenated compounds in the carcinogenicity training database. Ammonium perfluorooctanoic acid is fully fluorinated with 15 fluorine atoms. This degree of halogenation portends to a chemical reactivity that one would not expect in the chemical design of pharmaceutical compounds nor would be biosynthesized in plants. The inclusion of ammonium perfluorooctanoic acid in the control group, however, was still valid since this study was intended to test how well this predictive model performed with dietary constituents of both synthetic and natural origin.

Given the exceptional performance of this software, the QSAR modeling approach used in this study could be useful as a first-line indicator in predicting carcinogenic potential before a compound enters long-term rodent testing, or as a decision support modality in the safety review of food additives, botanicals, components of natural mixtures, and certain dietary constituents. An important consideration for this model is that the "high" prediction of carcinogenic potential by the discriminant analysis software does not provide a quantitative estimation of total risk of cancer in humans, nor identify target organ(s) where the occurrence of neoplasia could be anticipated. This is a limitation common to most computational predictions of carcinogenicity. Appropriate interpretation of the results should be based on coverage or representation of the chemical's molecular structure in the database. Built into the prediction, however, is an estimation of the probability that chronic oral exposure in rodents would have either a high or low potential for producing carcinogenic activity without regard to the dose required to produce such an effect. This probabilistic value generated by the QSAR modeling software is important in determining whether a prediction is valid or whether the software was unable to identify adequate molecular coverage.

It is well established that exposure to both naturally occurring and synthetic chemicals found in the diet represents the major portion of the total xenobiotic exposure to the body. As a group, natural products have been estimated to represent more than 99% of the chemicals humans ingest (Ames, 1998; Ames and Gold, 1998). Despite the wide range of naturally occurring chemicals present in the diet, there have been numerous reports describing, or at least partially characterizing, the activity of naturally occurring carcinogens derived from edible food, or herbal and botanical sources (National Resarch Council, 1982). Some well-known examples include heterocyclic amines (PhIP), cycasin (cycasin nut), ptaquilosin (braken fern), safroles (sassafras), pyrrolizidine alkaloids (*Senecio jacobaea*), and aristolochic acids (Virginia snakeroot). Each of these chemicals was tested using the rodent carcinogenicity module and the predictions were consistent with the experimental evidence for rodent carcinogenicity (Table 3). Likewise, well known compounds studied for protective effects against cancer, such as lipoic acid (spinach), epicatechin (green tea), and gallic acid (mango) would be expected to have a low carcinogenic potential, based on chronic animal bioassay testing and other relevant experimental evidence. The modeling results for these compounds bear out these expectations.

Categorically, the majority of chemicals screened in this study are found as integral components of foods that are relatively common in the human diet. Examples include capsaicin from hot peppers, lycopene from tomato, and ipomeamarone from sweet potato. Some of the other naturally occurring dietary chemicals tested in this study and only recently identified (such as *N*-benzylhexadecanamide) are found in less common food sources like the Peruvian plant food, maca, found only at high altitude in the Andes mountains (Valerio and Gonzales, 2005; Gonzales and Valerio, 2006). Several compounds originating from medicinal plants were tested because these materials are often administered orally for chronic periods of time, or may be considered for regulatory purposes as conventional food and often do not have long-term toxicological test data available. For example, under U.S. regulatory criteria, medicinal herbal teas are considered a conventional food. Therefore, added ingredients in herbal tea mixtures meet the statutory requirements of the Federal Food, Drug, and Cosmetic Act to qualify for safety evaluation as food ingredients. Regardless of whether the food ingredient is proposed to be generally recognized as safe (GRAS), or present as a direct food additive requiring premarket approval by the FDA, a validated means for assessing carcinogenic potential of the substance in absence of results from a chronic rodent bioassay would be useful.

A collective review of the actual safety profiles of chemicals screened in this study finds very low levels of exposure to chemicals with relatively weak carcinogenic activity in laboratory animals (e.g., isosafrole) and very low levels of exposure to chemicals with highly potent carcinogenic activity at all doses tested in multi-tissue sites (e.g., aristolochic acid I). Therefore, the actual hazards to human health posed by these

components of foods likely encompasses a full range from low to high. As supported by the high degree of positive predictivity (77%) and sensitivity (97%) of the computational modeling results, approximately 55% of the naturally occurring compounds presented in this study were found to be carcinogenic in laboratory animals. This observation is in contrast to the computational estimation by Klopman who found that 25% of 98 natural substances examined from edible plants were predicted to be rodent carcinogens (Rosenkranz and Klopman, 1990). There are a number of reasons for these differences. First, the compounds examined are not the same. Second, MCASE employs a different approach to predicting toxicity using molecular fragments and Lipinsky rules, and third, the MCASE database at the time of the study contained only 252 chemicals. The current version of MCASE (MC4PC version v. 1.60) contains over 1000 chemicals for building its predictions. The MDL-QSAR carcinogenicity module contains over 1200 chemicals. The most appropriate way to compare the predictive performance of MCASE and MDL-QSAR would be to conduct a modeling experiment on the set of compounds tested here using the most current version of the MCASE software and MDL-QSAR. We are currently undertaking this comparison. In the present study, the experimental evidence for rodent carcinogenic potential was assessed for all of the 142 natural and synthetic chemicals tested with MDL-QSAR, a research effort and approach that was not undertaken in the MCASE study of natural substances (Rosenkranz and Klopman, 1990). Future studies could include comparison of the predictive performance produced by multiple computational-based toxicological predictive systems (i.e., MDL-QSAR, MCASE, DEREK, TOPKAT, etc.) using the same external data set of chemicals.

Since the MDL-QSAR module is intended to make predictions on rodent carcinogenicity, the accuracy of the predictions for human cancer risk remains unknown. Therefore, the predictions made by this QSAR approach may serve only as an aid in human health risk assessment with the knowledge that the prediction is not a direct estimate of human carcinogenic risk for a single organic molecule. In the context of a new methodology for evaluating carcinogenic risk to naturally occurring dietary chemicals, the predictions made by this modeling approach should be used to estimate rodent carcinogenic potential within appropriate safety and risk analysis paradigms for assessing human health-based effects, and as a decision support tool so that priorities for further testing may be set. Clearly, there needs to be further evaluation with this QSAR module for its use with natural products such as those presented in this study. For example, additional testing with this QSAR module could include studies correlating pertinent epidemiological studies with MDL-QSAR predictions and further external validation tests modeling with other toxicological endpoints relevant to the induction of cancer such as genotoxic potential. The usefulness of this QSAR approach has been previously demonstrated to have utility as a tool for producing probabilistic estimates of carcinogenic risk in rodents for pharmaceutical compounds and candidate drugs under regulatory review (Contrera et al., 2003, 2005a).

However, despite these successes, some important limitations of MDL-QSAR for the estimation of carcinogenic risk based need to be highlighted. This approach, like most QSAR predictive models, does not distinguish between target organs for carcinogenicity, extrapolate risk from rodent to human, account for mechanistic considerations such as species- or sex-specificity, nor does it provide an estimation of dose or time of exposure (acute or chronic) necessary for induction of tumors. Moreover, the predictive screening method is unable to take into account synergistic or antagonistic effects that may occur with mixtures.

Many of these limitations and deficiencies also exist for rodent bioassay results in general, considering the poor trans-species site specificity comparing mouse and rat tumors and questions regarding the applicability of rodent tumor sites or rodent carcinogenic mechanisms to humans (Contrera et al., 1997).

The results of our study support the position of Ames and associates that rodent carcinogens are not rare, as these investigators estimate that half of all chemicals tested in standard high-dose animal cancer tests, whether naturally occurring or produced synthetically, are carcinogens (Ames, 1998; Ames and Gold, 1998). Approximately 55% of the naturally occurring chemicals with or without chronic bioassays that were screened with MDL-QSAR, had either direct experimental evidence of carcinogenic activity or other relevant data indicating a high risk for carcinogenicity such as Ashby–Tennant structural alerts.

It was not the objective of this study to re-evaluate the carcinogenic potential of these natural products found in the human diet. Rather, the objective was to explore the potential utility of the software in predicting the rodent carcinogenic potential of dietary chemicals.

The FDA's OFAS has found other methods that are useful with regard to assessing carcinogenic potential of untested substances, such as classification schemes for structural alerts developed by Ashby and Tennant (Ashby and Tennant, 1991) and Munro et al. (Munro et al., 1996). When used in combination, FDA/OFAS finds these classification schemes form a comprehensive list of structural alerts useful for addressing the diverse chemical universe (Bailey et al., 2005). The use of classification schemes for structural alerts to identify potentially hazardous agents is widely used by many agencies. For example, the classification scheme by Cramer et al. is currently used for safety assessments by the United Nation's Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) for determining the structural class of flavoring agents added to food (Cramer et al., 1978; Schrankel, 2004). However, outside of these structure-based methods, few experimental methods have explored predicting the carcinogenic potential of untested chemicals in high-throughput fashion. The widely used *in vitro Salmonella* mutagenicity assay and micronucleus test for genotoxic chemicals (McCann et al., 1975), cell-based assays for nongenotoxic carcinogenic predictions (LeBoeuf et al., 1996), animal "medium-term" bioassays (Ward and Ito, 1988), transgenic mouse (Tennant et al., 1995) and rat models (Spalding et al., 2000), and identification of specific

pathological endpoints from short-term animal toxicity studies have all made useful contributions in predicting chemical carcinogenicity (Allen et al., 2004).

Conclusions

This study is the first to demonstrate successful QSAR predictive modeling of naturally occurring carcinogens found in the human diet using an external validation test. The QSAR predictive modeling approach employed in this study was a high-throughput method employing discriminant analysis. Our findings are that this high-throughput approach could be very valuable in risk assessment and priority setting for the vast number of untested natural products, certain food additives and dietary constituents when it is used in combination with experimental evidence of rodent carcinogenic potential, structural alert classification schemes, and other aforementioned approaches in predictive toxicology.

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